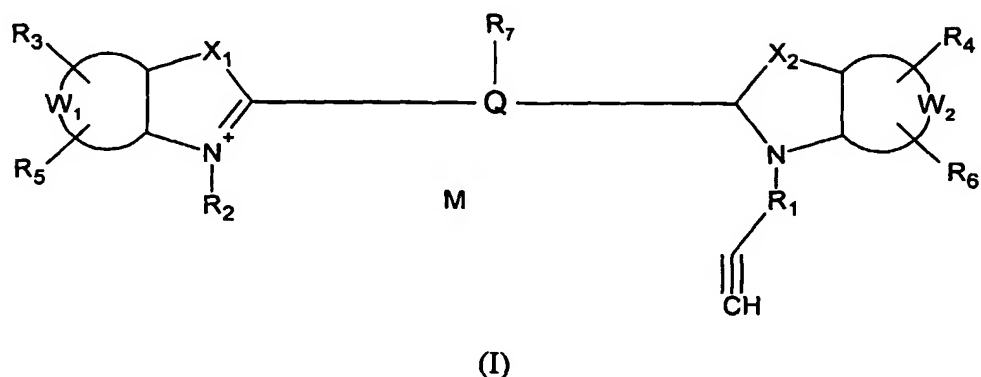


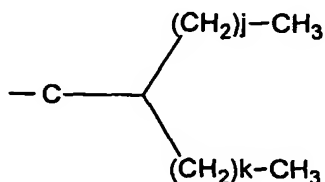
CLAIMS

1. A cyanine modified with an alkynyl-linker arm, having the following general formula (I), including the valence tautomers thereof:



wherein

$R_1$  is a linear, saturated or unsaturated alkyl chain, having from 1 to 30 carbon atoms, wherein one or more carbon atoms are each optionally substituted by a component independently selected from an oxygen or a sulfur atoms, a  $-NH-$  or a  $-CONH-$  group, or a cyclic 4-, 5- or 6- membered grouping of carbon atoms, aromatic or not aromatic, wherein one or more carbon atoms are each optionally substituted by a heteroatom independently selected from oxygen, sulfur, nitrogen and selenium;  $W_1$  and  $W_2$  are independently selected from a benzene ring and a naphthalene ring wherein one or more carbon atoms are optionally substituted by one or more heteroatoms selected from oxygen, sulfur, selenium and nitrogen, or one of  $W_1$  and  $W_2$  is absent, or both of them are absent;  $X_1$  and  $X_2$  are independently selected from the group consisting of  $-O-$ ,  $-S-$ ,  $-Se-$ ,  $-N-$ ,  $-C(CH_3)_2$ ,  $-CH=CH-$ ,  $-NH-$ , and



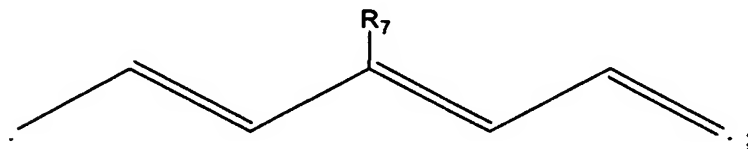
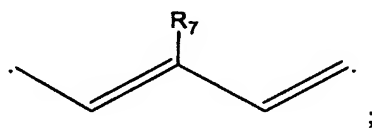
with  $j = 1-20$  and  $k = 1-20$ ;

$R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  are independently selected from hydrogen,  $-COOH$ ,  $-OH$ ,  $-NO_2$ ,  $-OCH_3$ ,  $-SO_3H$ ,  $-SO_3^-$ , and  $-R_8-Y$  wherein  $R_8$  is a linear, saturated or unsaturated alkyl chain, having from 1 to 30 carbon atoms, wherein one or more carbon atoms are each optionally substituted by a component independently

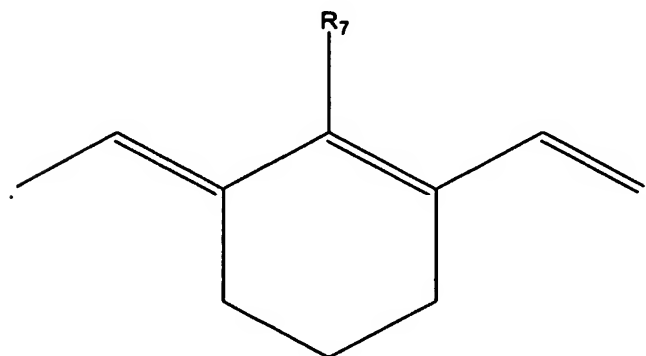
selected by an oxygen or a sulfur atom, a  $-NH-$  or a  $-CONH-$  group, or a cyclic 4-, 5- or 6- membered grouping of carbon atoms, aromatic or not aromatic, wherein one or more carbon atoms are each optionally substituted by a heteroatom independently selected from oxygen, sulfur, nitrogen or selenium, and wherein Y is selected from the group consisting of hydrogen, carboxyl, carbonyl, amino, sulphydryl, thiocyanate, isotyocianate, isocyanate, maleimide, hydroxyl, phosphoramidite, glycidyl, imidazolyl, carbamoyl, anhydride, bromoacetamido, chloroacetamido, iodoacetamido, sulphonyl halide, acyl halide, aryl halide, hydrazide, succinimidyl ester, hydroxysulfosuccinimidyl ester, phthalimidyl ester, naphthalimidyl ester, monochlorotriazine, dichlorotriazine, mono- or di- halide substituted pyridine, mono- or di- halide substituted diazine, aziridine, imidic ester, hydrazine, azidonitrophenyl, azide, 3-(2-pyridyldithio)-propionamide, glyoxal, aldehyde, nitrophenyl, dinitrophenyl, trinitrophenyl and  $-C\equiv CH$ ;

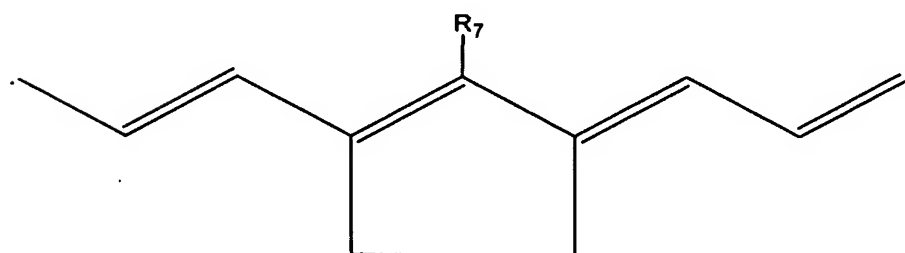
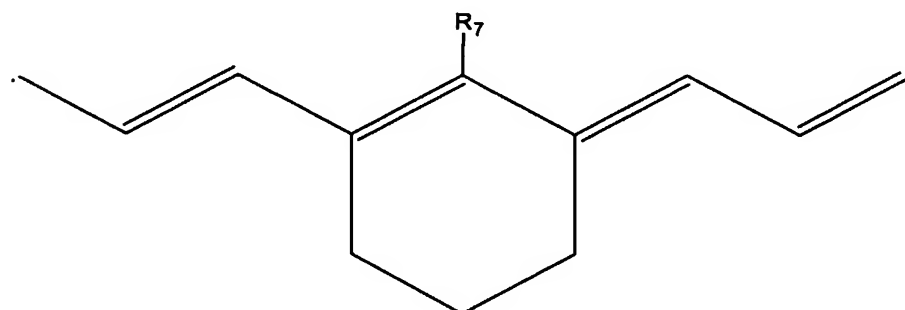
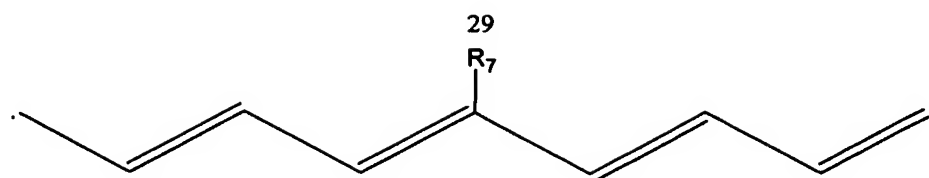
M is a counterion; and

Q is a polymethinic chain selected from:



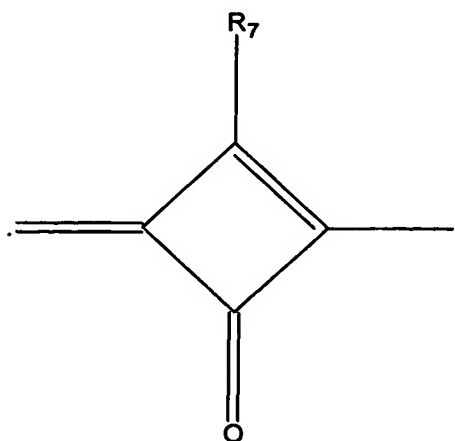
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or



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wherein R<sub>7</sub> is selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, phenoxy, thiophenoxy, anilino, cyclohexylamino, piridine, -R<sub>8</sub>-Y, -O-R<sub>8</sub>-Y, -S-R<sub>8</sub>-Y, -NH-R<sub>8</sub>-Y, wherein R<sub>8</sub> e Y are as defined above, and aryl optionally substituted by one or more substituents independently selected from the group consisting of -SO<sub>3</sub>H, carboxyl (-COOH), amino (-NH<sub>2</sub>), carbonyl (-

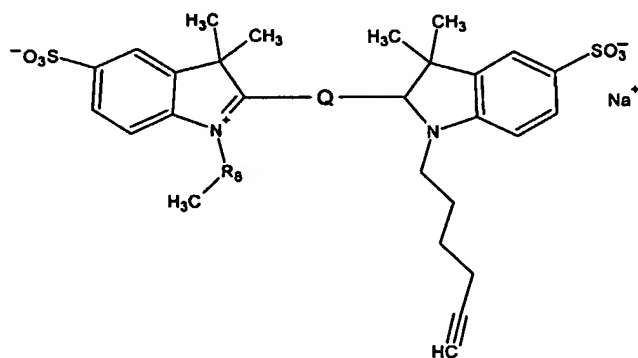
CHO), thiocyanate ( $-\text{SCN}$ ), isothiocyanate ( $-\text{CNS}$ ), epoxy and  $-\text{COZ}$  wherein Z represents a leaving group.

2. The cyanine according to claim 1, wherein said leaving group is selected from the group consisting of  $-\text{Cl}$ ;  $-\text{Br}$ ;  $-\text{I}$ ;  $-\text{OH}$ ;  $-\text{OR}_{11}$ ;  $-\text{OCOR}_{11}$ , wherein  $\text{R}_{11}$  is linear or branched alkyl having from 1 to 4 carbon atoms;

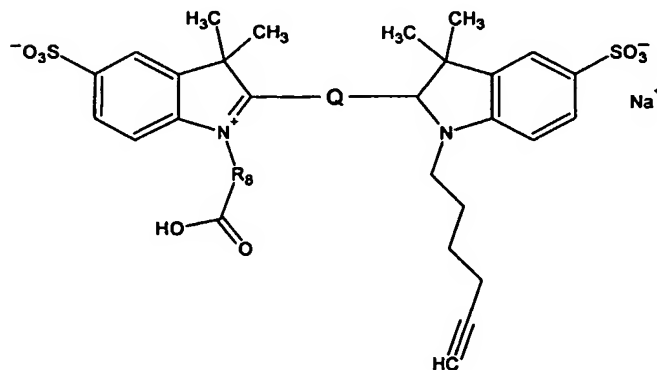
$-\text{O}-\text{CO}-\text{Ar}$ , wherein Ar is aryl optionally substituted;  $-\text{O}-\text{CO}-\text{Het}$ , wherein Het is selected from succinimide, sulfosuccinimide, phthalimide and naphthalimide;  $-\text{NR}_{22}\text{R}_{33}$ , wherein  $\text{R}_{22}$  and  $\text{R}_{33}$  are each independently linear or branched alkyl having from 1 to 10 carbon atoms.

3. The cyanine according to claim 1 or 2, wherein one of  $\text{R}_2$ ,  $\text{R}_3$ ,  $\text{R}_4$ ,  $\text{R}_5$  and  $\text{R}_6$  is  $-\text{R}_8-\text{Y}$ , wherein Y is different from H and from  $-\text{C}\equiv\text{CH}$ .

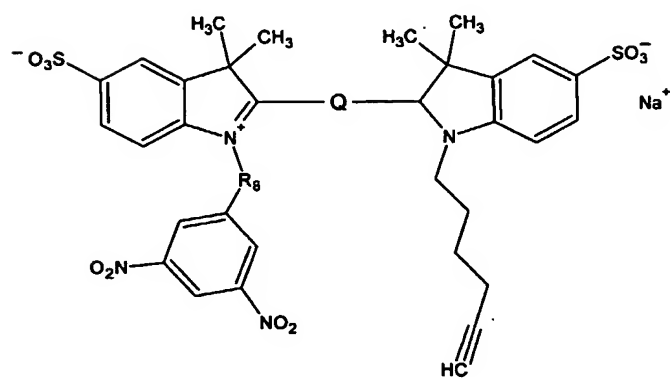
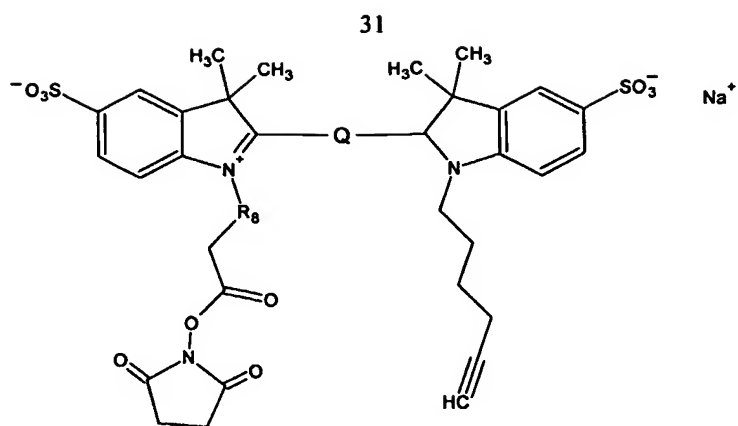
4. The cyanine according to claim 3 selected from the group consisting of:



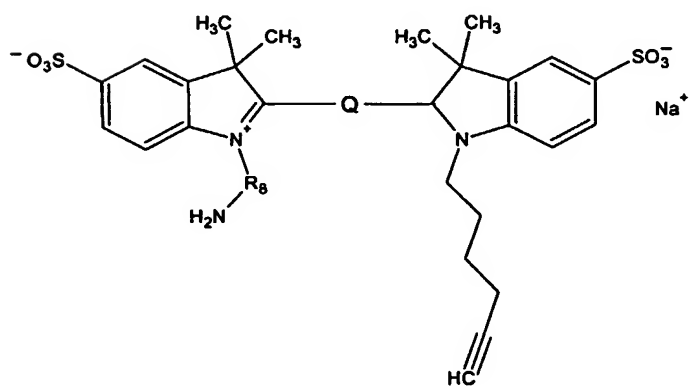
Formula (Ia)

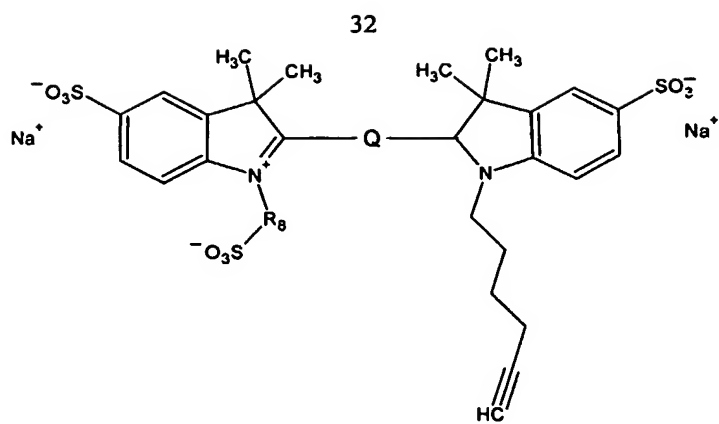


Formula (Ib)

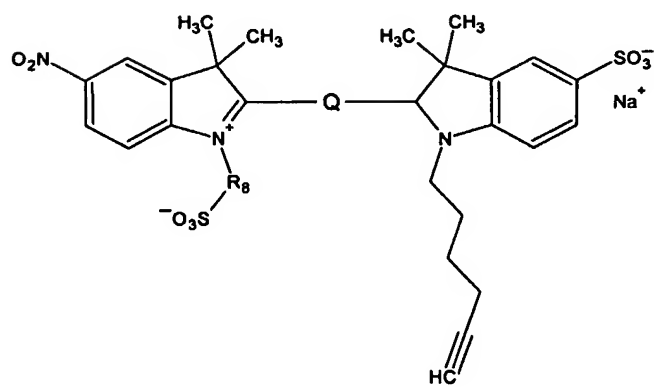


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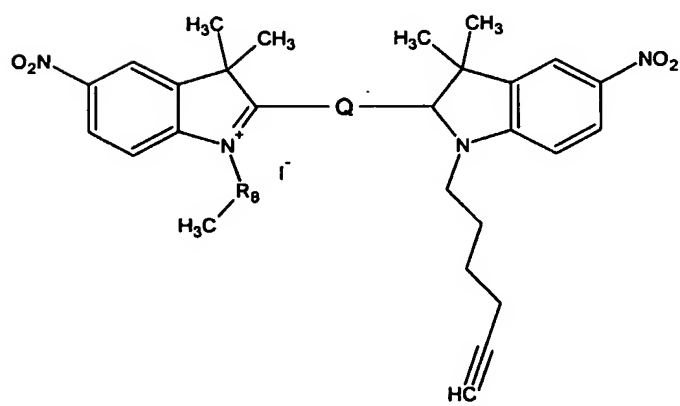




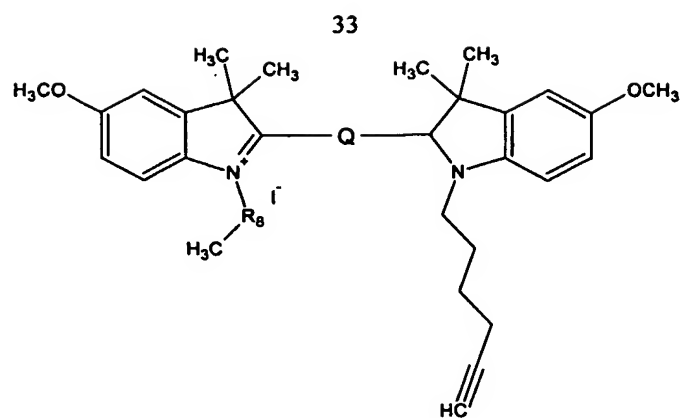
Formula (If)



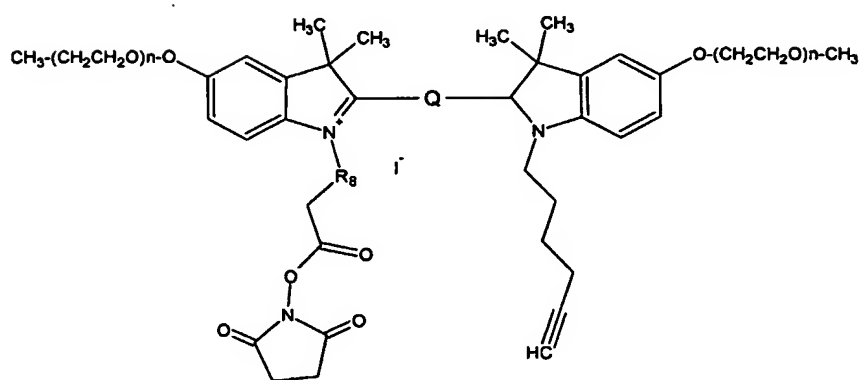
Formula (Ig)



Formula (Ih)

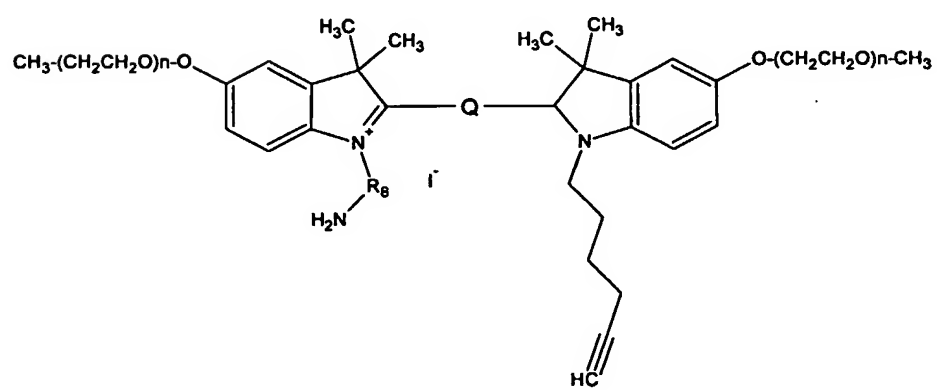


Formula (Ii)

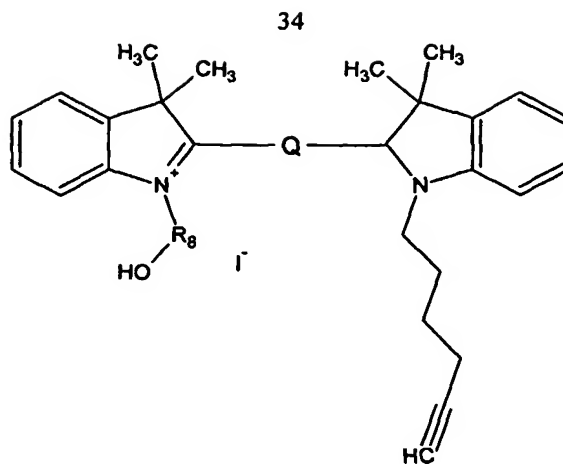


Formula (II)

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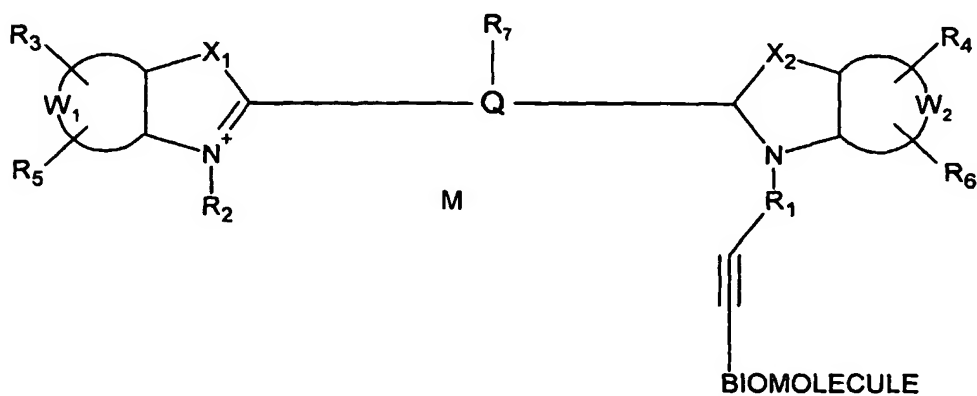
Formula (Im)



Formula (In),

wherein Q and R<sub>8</sub> are as defined in claim 1 and n is an integer between 1 and 100.

- 5        5. The cyanine according to any of the claims 1 to 4, conjugated through the linker arm -R<sub>1</sub>-C≡CH with a biomolecule, said conjugated cyanine being represented by the general formula (II), including the valence tautomers thereof:

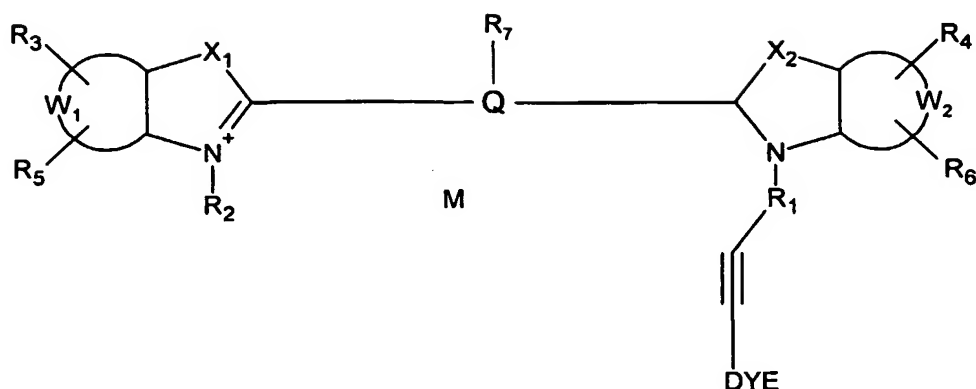


10        wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, W<sub>1</sub>, W<sub>2</sub>, X<sub>1</sub>, X<sub>2</sub>, Q and M are as defined in claim 1.

6. The cyanine according to claim 5, wherein said biomolecule is selected from the group consisting of nucleotides, nucleosides, oligonucleotides, nucleic acids, peptides and proteins.

- 15        7. The cyanine according to any of the claims 1 to 4, conjugated through the linker arm -R<sub>1</sub>-C≡CH with a second fluorescent dye, said second fluorescent dye being capable of emitting fluorescence at wavelengths at which the cyanine is capable of absorbing, or said fluorescent dye being capable of absorbing at wavelengths at which the cyanine is capable of emitting, said cyanine conjugated with a second fluorescent dye being represented by the general formula (III), including the valence tautomers thereof:
- 20

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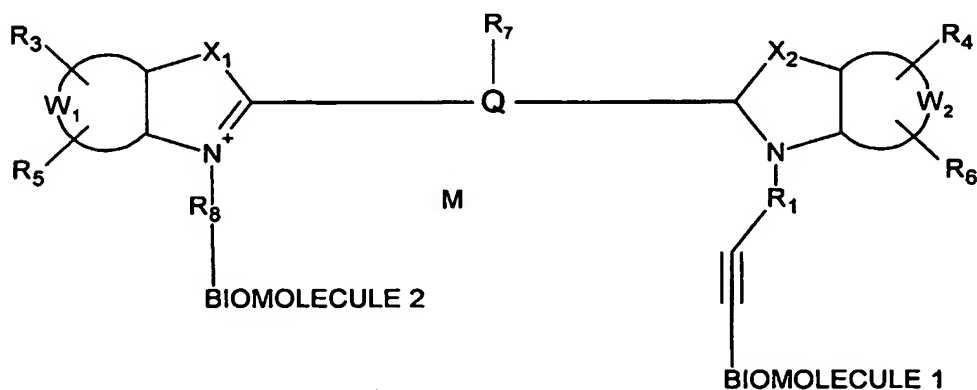
(III)

wherein  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $W_1$ ,  $W_2$ ,  $X_1$ ,  $X_2$ ,  $Q$  and  $M$  are as defined in claim 1.

8. The conjugated cyanine according to claim 7, wherein said second fluorescent dye is N,N'-Difluoroboryl-1,9-dimethyl-5-(4-iodophenyl)-dipyrrin.

9. The conjugated cyanine according to claim 7, wherein said second fluorescent dye is a transition metal complex with at least one heterocyclic nitrogen-containing ligand.

10. The cyanine according to claim 3, conjugated through the linker arm  $-R_1-C\equiv CH$  with a first biomolecule selected from the group consisting of nucleotides, nucleosides, oligonucleotides, nucleic acids, peptides, proteins, vitamins and hormones, and through the linker arm  $-R_8-Y$  with a second equal or different biomolecule selected from the group consisting of nucleotides, nucleosides, oligonucleotides, nucleic acids, peptides, proteins, vitamins and hormones, said cyanine conjugated with a first and a second biomolecule being represented by the general formula (IV):

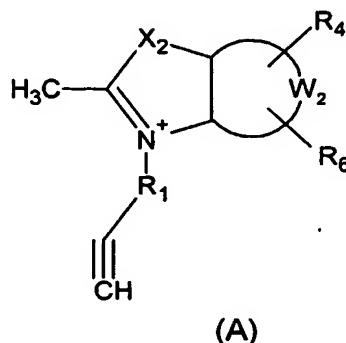


(IV)

wherein  $R_1$ ,  $R_3$ ,  $R_4$ ,  $R_5$ ,  $R_6$ ,  $R_7$ ,  $R_8$ ,  $W_1$ ,  $W_2$ ,  $X_1$ ,  $X_2$ ,  $Q$  and  $M$  are as defined in

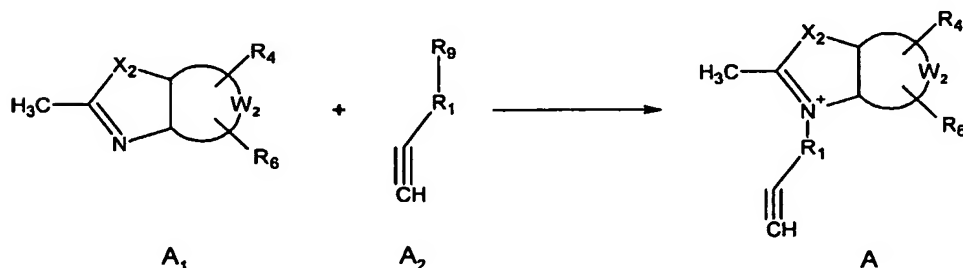
claim 1.

11. Intermediate for preparing a cyanine modified with an alkynyl linker arm of formula (I) as defined in claim 1, said intermediate being represented by the general formula (A):



wherein R<sub>1</sub>, R<sub>4</sub>, R<sub>6</sub>, X<sub>2</sub>, W<sub>2</sub> are as defined in claim 1.

12. A method for preparing an intermediate of formula (A) as defined in claim 11, comprising the step of reacting a nitrogen containing heterocyclic system of formula A<sub>1</sub> with a molecule containing a triple bond of formula A<sub>2</sub> to form a quaternary ammonium salt of formula A:



15 wherein X<sub>2</sub>, R<sub>1</sub>, R<sub>4</sub>, R<sub>6</sub> and W<sub>2</sub> are as defined in claim 1, and R<sub>9</sub> is selected in the group consisting of iodine, chlorine, bromine, OH, sulfate and tosylate.

13. The use of a cyanine according to any of the claims 1 to 4 as a fluorescent marker for biomolecules or as a quencher.